UNCLASSIFIED

AD 418252

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA

UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

SAM-TDR-63-49

CATALOGED BY DDC
AS AD No. 418252

64-5

RAPID, ACCURATE ANALYSIS OF BLOOD LACTATE

TECHNICAL DOCUMENTARY REPORT NO. SAM-TDR-63-49

June 1963

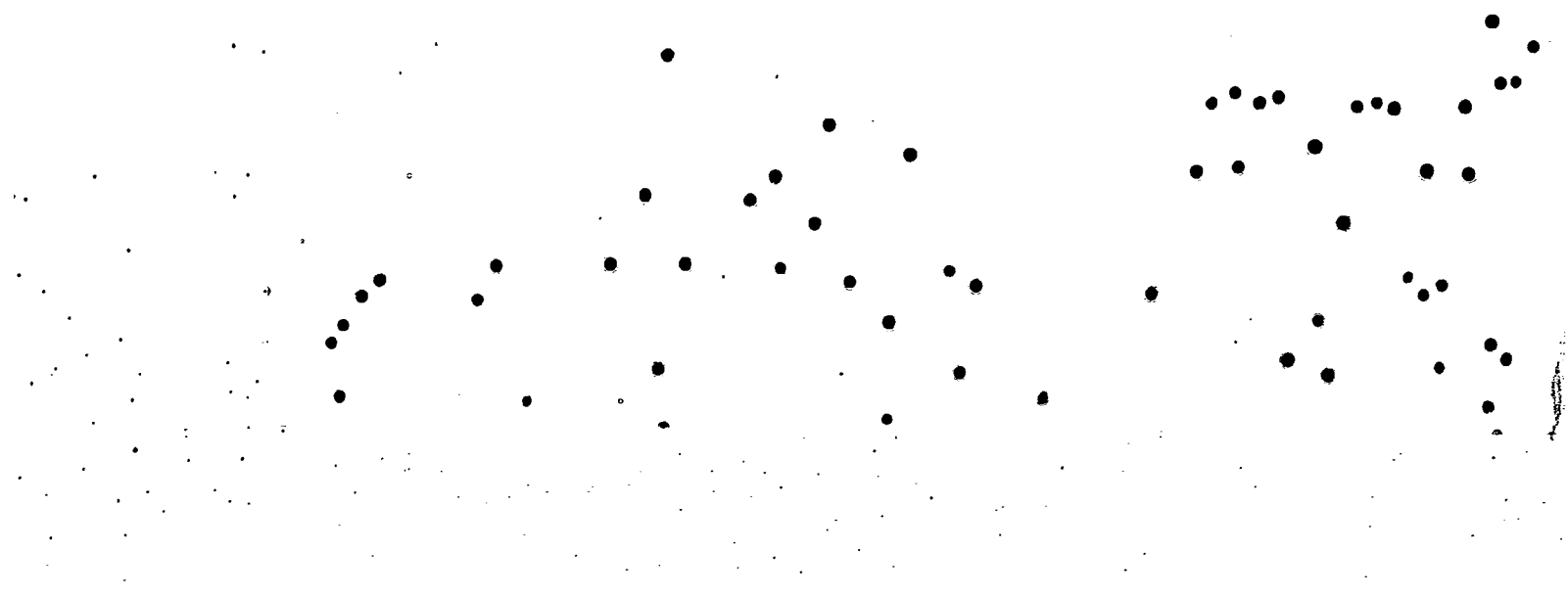
USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

SEP 23 1963

Task No. 775801

Qualified requesters may obtain copies of this report from ASTIA. Orders will be expedited if placed through the librarian or other person designated to request documents from ASTIA.

When U. S. Government drawings, specifications, or other data are used for any purpose other than a definitely related government procurement operation, the government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.



FOREWORD

This report was prepared by the following personnel in the Physiology Section:

JAMES P. ELLIS, JR., M.A.
STEPHEN M. CAIN, Ph.D.
EDGAR W. WILLIAMS, B.A.

ABSTRACT

A method is described for the enzymatic analysis of blood L-lactic acid. The method is based on existing methods, but incorporates certain unique features which permit a rapid and precise analysis of a large number of samples. One of the more important features is the use of a simplified procedure for correcting the problematic drift of the unstable reaction mixture. In addition, conditions for the enzymatic activity were established which facilitate complete oxidation of lactate in 30 to 35 minutes. A comparative analysis of several blood filtrates, using the present method versus a well-known chemical method, as well as recovery analyses using the two methods, establishes the validity of the present test system.

This technical documentary report has been reviewed and is approved.

ROBERT B. PAYNE
Colonel, USAF, MSC
Chief, Operations Division

RAPID, ACCURATE ANALYSIS OF BLOOD LACTATE

1. INTRODUCTION

On the basis of numerous reports within recent years, the rather time-consuming and nonspecific analysis of lactic acid by so-called chemical methods has been virtually replaced by enzymatic methods (1-8). While these reports clearly establish the general preference of the enzymatic method, methodologic differences between them indicate that no conventional or widely accepted procedure has been established. Thus, there does not seem to be a preferred protein precipitant, pyruvate-trapping agent, end product (DPNH, dye), correction for an unstable reagent blank, pH of reaction mixture, nor optimum concentration of the various reactants. Olson (7) recently reported the optimum conditions for an enzymatic method, but certain aspects of the method seem worthy of further consideration and perhaps modification.

The present report describes an enzymatic method which combines certain features of existing methods and introduces innovations to form a simplified, rapid, and accurate measure of blood lactate. The analysis of 24 blood samples, in duplicate, and with an appropriate number of blanks and calibration standards, can be readily accomplished in four hours.

2. METHODS

Solutions, reagents, etc.

Glycine-hydrazine buffer. A liter of pH 9.0 buffer was prepared by first dissolving 37.5 gm. of glycine in approximately 800 ml. of distilled water. After adding 13.5 ml. of hydrazine (1.011 sp. gr., 95%), the solution was thoroughly mixed and the pH adjusted to 9.0 with 2.5 N sodium hydroxide. This buffer contains 0.5 M glycine and 0.4 M hydrazine when diluted to 1 liter with distilled water, and is stable for at least two weeks when stored under refrigeration.

Received for publication on 5 April 1948.

Lactic dehydrogenase. An ammonium sulfate suspension of crystalline muscle lactic dehydrogenase (41.8 mg. enzyme protein per milliliter, 61.4 units per milligram) was diluted 1:5 with distilled water. While the dilute preparation will retain its activity for several weeks when stored in a refrigerator, a volume in slight excess to that required for the day was prepared.

Diphosphopyridine nucleotide. A 30 mg./ml. solution of DPN was prepared fresh each day.

L-*Lactic acid.* A 40% stock sodium lactate solution was standardized by chemical analysis (9) using recrystallized lithium lactate standards. An intermediate standard was prepared from the stock solution which contained 2.00 mg. L-lactic acid per milliliter. A set of five calibration standards, ranging from 10 to 50 mg. per 100 ml., was prepared at frequent intervals from the intermediate standard.

p-Hydroxydiphenyl reagent. p-Hydroxydiphenyl (1.5 gm.) was dissolved in 5% NaOH (10 ml.) and diluted to 100 ml. with distilled water. This reagent will remain stable for several weeks when stored in an amber bottle and refrigerated.

Cupric sulfate (4% and 20%).
Calcium hydroxide (powder).
Sodium hydroxide (2.5 N).
Sulfuric acid (concentrated).
Perchloric acid (6% w/v).
Trichloroacetic acid (10% w/v).

Preparation of protein-free filtrates

Three methods for the collection of blood were examined. The first method consisted

of allowing approximately 3.0 ml. of dog blood to flow from a femoral arterial catheter directly into a weighed test tube containing 5.0 ml. of 10% trichloroacetic acid (TCA). The exact volume of blood in the filtrate was then computed on the basis of the weight of a precisely measured 3.0 ml. volume of a representative blood sample. In the second method, a 3.0 ml. pipet was connected directly to the femoral catheter, and the precise volume of blood was immediately added to 5.0 ml. of 10% TCA. The third method consisted of drawing the blood from the catheter with a syringe, transferring the blood to a test tube, and then transferring a 3.0 ml. volume into 5.0 ml. of 10% TCA. Contents of all tubes were promptly and vigorously mixed and then centrifuged for 20 minutes at 3,000 r.p.m.

A limited number of filtrates were prepared, using an equal volume of blood and 6% perchloric acid (PCA). The acidified samples were centrifuged for 20 minutes at 3,000 r.p.m., decanted, and the supernatant centrifuged again.

TCA mixtures of the previously mentioned lactate calibration standards were freshly prepared according to the procedure described above. The procedural blank consisted of adding 3.0 ml. of distilled water to either 5.0 ml. of 10% TCA or 3.0 ml. 6% PCA.

Enzymatic analysis of lactate

The method herein described for the measurement of blood lactate is similar to the procedural instructions supplied with the Boehringer Test Combination (6). It consists of adding 2.0 ml. of glycine-hydrazine buffer to a series of test tubes, the number depending on the number of specimens to be analyzed. The first six sets of duplicate test tubes were reserved for TCA filtrates representing 0 (blank), 10, 20, 30, 40, and 50 mg. L-lactic acid per 100 ml. Because of a time limitation in a subsequent phase of the analysis, a maximum of 25 blood samples, in duplicate, can be processed at a time. In most cases, a 0.1 ml. volume of the filtrate resulted in a lactate value falling within the range of accuracy. However, for samples suspected of having a lactate content in excess of 50 mg. per 100 ml., it was necessary to dilute the filtrate. Rather than diluting the filtrate prior to the addition to the buffer, we found it convenient to add 0.05 ml. of the blood filtrate and 0.05 ml. of the blank filtrate to the buffer. The addition of the latter was essential for the maintenance of standard test conditions within the reaction mixture. Exactly 0.03 ml. of the dilute lactic dehydrogenase (LDH) preparation was added to each tube, and the contents thoroughly mixed. Up to this point in the procedure, the time factor for the addition of components was not considered too critical. However, at a precisely noted time, 0.2 ml. DPN was added to the set of duplicate blanks, and thereafter added consecutively to the remaining sets of tubes at exactly 2-minute intervals. The reaction mixture was again mixed and then allowed to remain at room temperature (26° C.) during the incubation interval. After the lapse of precisely 60 minutes, optical density readings were obtained, using a Beckman model DU spectrophotometer at a wavelength of 340 mμ. and a slit of 0.3 mm. The 60-minute incubation period was easily maintained for all samples with the allowance of the 2-minute interval between, for the filling of cuvettes and the reading and recording of optical density values. One-half (0.5) ml. silica cuvettes, 10 mm. light path, were used and all samples were read against distilled water.

For the calculation of lactate concentration, the average optical density of the blank was subtracted from all other optical density values. The calibration curve was constructed from the net absorbance values of the standard lactate filtrates, and the apparent lactate concentration of the blood filtrate was read off directly as milligrams of lactic acid per 100 ml. blood.

The true lactic acid content was then obtained by multiplying the apparent value by a factor determined by the following equation:

$$\text{Factor} = \frac{(V_1 \cdot W \cdot F) + V_2}{V_1 + V_2}$$

where V_1 is the volume of blood added to TCA (3.0 ml.), V_2 is the volume of TCA (5.0 ml.), W is the weight of 1.0 ml. blood, and F is the

liquid fraction of blood. Assuming a normal weight of 1.06 gm./ml. and a blood water fraction of 80%, the factor becomes 0.942.

The analytic procedure, described above, was also carried out—a double volume of each component being used, in an attempt to eliminate the use of micropipets and microcuvettes.

Colorimetric (chemical) analysis of lactate

A slight modification of the Barker and Summerson method (9) was used as the basis for assessing the performance of the enzymatic method. The modification consisted of placing 0.6 ml. of the TCA filtrate in a 15 ml. conical centrifuge tube and adding 1.0 ml. 20% $CuSO_4$ and 8.4 ml. distilled water. Procedural blanks and calibration standards (representing 10, 20, and 30 mg. per 100 ml.) were processed with each group of blood filtrates, and all samples were read against the procedural blank.

3. RESULTS

Instability of reaction mixture

The data presented in figure 1 clearly demonstrate the need for a careful and continuous control over the reagent blank. A typical blank obtained with the present test system (curve a) has an initial marked increase in absorption, followed by a slight, but progressive, increase throughout the remainder of the incubation period. While not shown in figure 1, this rate of increase (approximately 0.001 O.D. units each 2 minutes) was maintained throughout a second 60-minute interval.

That this increase in absorbance is DPN-dependent is illustrated by curve b, figure 1. The omission of LDH from the reaction mixture, with the addition of an appropriate volume of water, produced a drift very similar to that of the complete mixture. On the other hand, omission of DPN (curve c) caused the reaction mixture to have even less absorption than the reference (water). Thus, in order to have a precise control over the side reaction between DPN and the buffer (hydrazine) as well as over the enzymatic reaction, DPN should be the activating component.

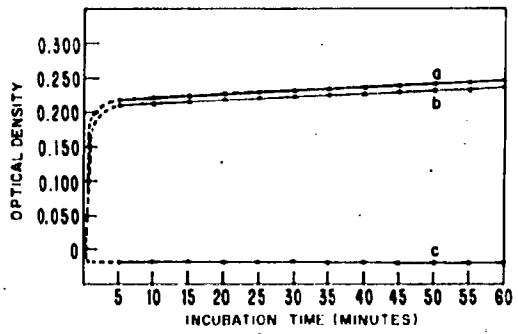


FIGURE 1

Instability of reaction mixture: **Curve a**—*complete reaction mixture (reagent blank);* **curve b**—*reaction mixture minus LDH; and* **curve c**—*reaction mixture minus DPN.*

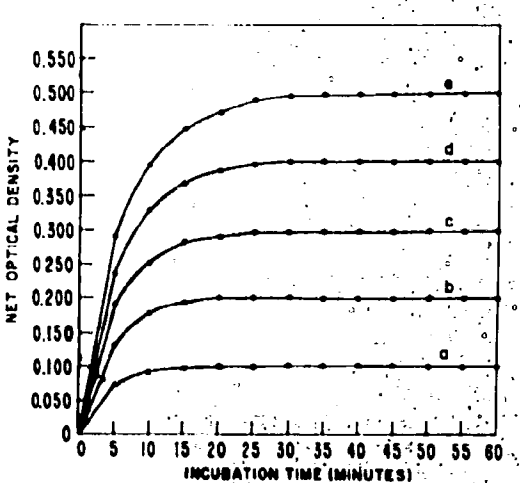


FIGURE 2

DPNH formation from oxidation of L-lactate: **Curves a** *through* **e** *represent, respectively, lactate concentrations of 0.0179, 0.0358, 0.0537, 0.0716, and 0.0895 μM. per milliliter of reaction mixture (corresponding to blood levels of 10, 20, 30, 40, and 50 mg. lactate acid per 100 ml.).*

Calibration data

The rates of DPNH formation from standard lactate solutions, by use of the present test system, are shown in figure 2. As might be expected, DPNH formation was complete in a shorter period of time at the lower lactate

concentrations. Even at the highest concentration tested, however, the formation was complete within 35 minutes. Stability of net absorbance values was maintained for the remainder of the incubation period. From these data, it is apparent that absorbancy readings could be made as early as 35 minutes without a loss in sensitivity or accuracy.

It is also apparent from the data in figure 2 that linearity exists throughout the lactate range tested. As a coincidental finding, net optical density values of 0.100, 0.200, 0.300, 0.400, and 0.500 corresponded to 10, 20, 30, 40, and 50 mg. L-lactate per 100 ml., respectively. The range of lactate concentration could be extended beyond the 50 mg. % level, but a slight loss of precision would result owing to the error of reading high optical density values. For example; with the relationship of an increase of 0.100 net O.D. units for each 10 mg. % increment, and with a reagent blank absorption of 0.250, a sample containing 60 mg. % would have an uncorrected O.D. of 0.850.

Establishment of method

A number of exploratory experiments were carried out before the present test system was established. Various combinations of molar strength of the buffer (0.2 to 0.5 M), pH of the buffer (9.0 to 10.0), and the pyruvate trap (semicarbazide, hydrazine, and hydrazine sulfate) were tested. An attempt was made, also, to use a smaller quantity of LDH and DPN.

The use of a weaker buffer (0.2 M glycine, 0.2 M hydrazine) caused a reduced rate of DPNH formation, which was attributed to the increase in acidity of the reaction mixture resulting from the addition of the acidified filtrate. The pH of the weakly buffered reaction mixture changed approximately 1.10 units, whereas the pH of the reaction mixture containing a stronger buffer (0.5 M glycine, 0.4 M hydrazine) fell only about 0.10 unit upon the addition of the filtrate. Whereas the use of a stronger buffer favored DPNH formation, there was an increase in background absorption due to the greater quantity of the trapping agent used.

A more alkaline buffer (pH 10.0) caused an additional increase in background absorption and resulted in a slight reduction of net O.D. values.

Equimolar concentration of hydrazine and hydrazine sulfate gave approximately the same background absorption, but both were less than that obtained with semicarbazide. Additional experimentation revealed that 0.4 M hydrazine was an adequate trap for pyruvate formed in the in vitro oxidation of lactate, as well as for excessive amounts of preformed pyruvate.

The use of a more dilute LDH preparation (4.18 mg. enzyme protein per milliliter) resulted in a narrowing of the useful range of accuracy; that is, the calibration curve was no longer linear beyond 40 mg. % lactate.

The use of a more dilute DPN solution (20 mg./ml.) reduced the background absorption and provided a linear calibration curve through 50 mg. %, which was slightly lower than the curve obtained with a 30 mg./ml. solution. An examination of the DPNH formation rate, however, indicated instability throughout the incubation period. The formation proceeded similarly to that shown in figure 2, but after reaching the maximum value within 20 to 35 minutes, a slight, but progressive, loss of net absorbance resulted. This loss was proportional to the concentration of lactate, thereby accounting for the linear calibration curve. Use of the higher concentration of DPN prevented the loss of net absorbance and accounted for the slightly higher calibration curve.

Calibration curves for standard lactate solutions prepared in 10% TCA and 6% PCA are shown in figure 3. While the standards shown correspond to a blood lactate range of 10 to 50 mg. %, the lactate concentration is expressed as micromoles per milliliter of reaction mixture since the proportion of acid:sample is different for the two types of filtrates. It is readily apparent that higher net O.D. values were obtained with PCA filtrates. This difference in response was due solely to the greater dilution of the sample with TCA. When

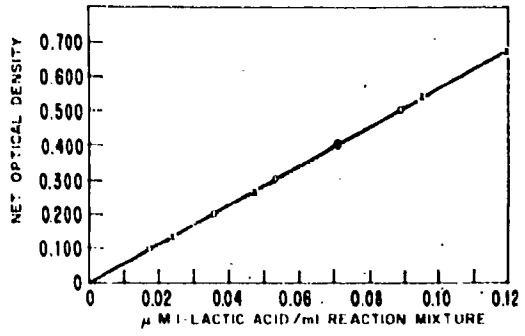


FIGURE 3

Calibration curve: Lactate concentrations of 10, 20, 30, 40, and 50 mg./100 ml. diluted 3:5 with 10% TCA (x x) or 1:1 with 6% PCA (o o).

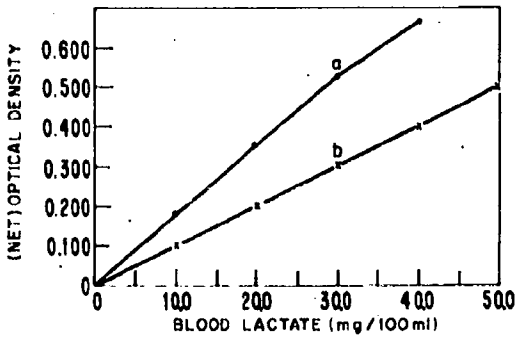


FIGURE 4

Calibration curves for chemical (curve a) and enzymatic (curve b) methods.

expressed as micromoles of lactate per milliliter of reaction mixture, the two types of filtrates gave an identical calibration curve.

Enzymatic vs. chemical analyses

In comparing the performance characteristics of the present enzymatic method with the Barker and Summerson chemical method, consideration was first given to the calibration curves obtained by the two methods. According to typical calibration curves given in figure 4, the enzymatic method (curve b) covers a wider range of blood lactate. Linearity ceases beyond 30 mg. % with the chemical method (curve a), whereas it extends to 50 mg. % with the enzymatic method. If, on the other hand, the calibration curves had been expressed in terms of micrograms per milliliter of test solution, rather than as milligrams percent, it would have been seen that the chemical method was about nine times more sensitive than the enzymatic method. For example, an O.D. of 0.360 by the chemical method (20 mg. %) would correspond to 0.65 μg. per milliliter of the concentrated H_2SO_4 solution, whereas an identical O.D. by the enzymatic method would require 5.57 μg. lactate per milliliter of reaction mixture (36 mg. %).

The recovery of L-lactate added to whole blood, as measured by the chemical and enzymatic methods, is illustrated in figure 5.

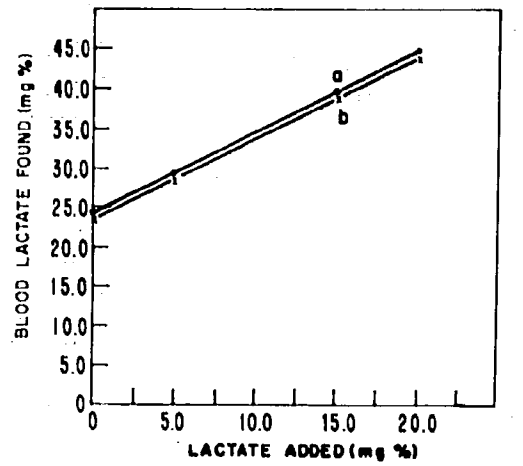


FIGURE 5

Recovery of lactate by enzymatic (curve a) and chemical (curve b) methods.

The two methods not only agreed as to the initial level of blood lactate, but they also indicated an approximate 100% recovery of added lactate in all cases.

The results obtained from the comparative analysis of 24 samples of normal dog blood by the chemical and enzymatic methods are presented in table I. The mean value obtained by the chemical method was 2.4% higher than that obtained by the enzymatic method. A statistical analysis of these data, however,

TABLE I

Chemical vs. enzymatic analysis of blood filtrates

Blood sample	Blood lactate (mg. %)		
	Chemical	Enzyme	Chemical-enzyme difference
1	8.0	7.8	0.2
2	8.2	7.7	0.5
3	9.1	9.3	0.2
4	9.9	9.5	0.4
5	9.9	10.6	0.7
6	10.1	9.8	0.3
7	10.3	9.2	1.1
8	10.6	10.6	0
9	10.6	10.3	0.3
10	10.7	10.1	0.6
11	11.5	10.5	1.0
12	11.7	11.0	0.7
13	12.3	11.4	0.9
14	13.8	13.1	0.7
15	15.5	14.0	1.5
16	15.5	15.2	0.3
17	15.9	16.0	0.1
18	16.0	14.0	2.0
19	16.4	15.4	1.0
20	16.4	17.0	0.6
21	17.1	17.6	0.5
22	17.4	17.5	0.1
23	19.6	20.1	−0.5
24	23.6	25.0	−1.4
Mean	13.3	13.0	0.3

TABLE II

Blood collection technics

Blood sample	Blood lactate (mg. %)		
	Collection method 1	Collection method 2	Collection method 3
1	15.4	14.6	26.7
2	14.8	15.1	29.8
3	33.2	33.0	44.4
4	15.6	16.3	*
5	16.1	16.8	
6	16.4	16.5	
7	18.9	16.6	
8	18.0	18.9	
9	19.7	19.3	
10	21.1	21.1	
11	21.7	21.6	
12	22.6	23.3	

*Samples 4 to 12 were not collected by method 3 because, by this time, it was considered a fruitless approach.

failed to establish this difference as significant. The mean difference of 0.3 mg. % did not differ significantly from zero ($P > .05$).

Blood collection technics

While not directly related to the establishment or assessment of the present method, the results given in table II illustrate the importance of blood collection technics. It is readily apparent that meaningful lactate values can be obtained only if the blood is promptly added to the acid precipitant. The slight delay encountered in drawing the blood into a syringe, with transfers into a test tube and a pipet prior to acidification, results in lactate values which are substantially higher than the presumably "true" level. Inasmuch as collection methods 1 and 2 gave comparable results, the latter method was considered the most appropriate since it required less time and effort and would seem to have wider application.

4. DISCUSSION

In our attempt to replace the previously employed Barker and Summerson method with an enzymatic method, we were unable to obtain the required performance characteristics with existing methods. Our specific need was to have a method precise enough to measure small changes in blood lactate concentration (e.g., brain A-V difference), yet practical enough to permit the analysis of a rather large number of samples on a routine basis.

The one factor which has hindered a rapid and precise measurement is the instability of the reaction mixture. Under the present test conditions, a drift of 0.001 O.D. units each 2 minutes would cause an error of about 0.5 mg. % over a 10-minute period. While the need for having a procedural blank with each set or series of determinations has been mentioned by other investigators, a limit as to the size of the set or series has not apparently been established. For example, instructions which accompany a commercially available assay kit (6) call for the absorbancy of a series of test samples to be read against a reagent blank, with the time after DPN addition being exactly equal for test and blank

samples. When consideration is given to the additional time required for the filling of cuvettes, etc., the series of test samples must necessarily be small (duplicate analysis of 1 to 2 specimens). Consequently, the reading of unknown and standard specimens against a "true" procedural blank would require a number of reagent blanks, and would, therefore, be more time-consuming and necessitate the use of more glassware and expensive reagents.

The use of a stable media, such as distilled water, as the reference for all blank and test absorbancy readings is not novel. While not specifically indicated, Olson (7) apparently utilized such a system. The present method, however, provides a suitable correction factor which does not necessitate multiple absorbancy readings of the same sample with the subsequent use of a formula for computing the net absorbance.

A seemingly more important difference between the present method and that of Olson is that the latter method utilizes the addition of LDH, rather than DPN, as the activator for the test system. While the addition of either component would serve to activate the system, there is good reason to believe that a better control over the drift factor would be obtained by using DPN as the final additive. The present results clearly demonstrate that the addition of LDH caused neither an immediate increase in absorbancy of the buffer nor a progressive increase over the following 60-minute incubation period. On the other hand, the addition of DPN to the buffer produced an initial and final absorbance similar to that found for the complete reaction mixture. Thus, the use of Olson's method would seem to require a careful control of time over two experimental periods—that is, the period between DPN and LDH additions, as well as the 60- or 120-minute interval following the addition of LDH.

The addition of LDH prior to DPN seems desirable for still another reason. Since the addition of LDH to the buffer resulted in a completely stable mixture, consolidation of these two reagents into a single solution is feasible. The use of a single reagent would eliminate a time-consuming and delicate pipetting step (i.e., 0.03 ml. LDH).

For several reasons, the use of the 0.5 M glycine–0.4 M hydrazine buffer at a pH of 9.0, as recommended by the enzyme kit method, seemed superior to the more alkaline (pH 10.0) and weaker (0.2 M) buffer (glycine-semicarbazide) recommended by Olson. From a practical standpoint, the use of a stronger buffer permits the direct use of acid filtrates. In sharp contrast, Olson recommends that the filtrate be carefully adjusted to pH 10.0 prior to being added to the buffer. Thus, the use of a stronger buffer eliminates a time-consuming procedural step. A pH 9.0 buffer was selected primarily because it gave less background absorption than the pH 10.0 buffer. Likewise, hydrazine and semicarbazide seemed equally effective as the pyruvate-trapping agent, but the hydrazine was found to produce less background absorption.

The enzyme protein concentration recommended by Olson was found necessary with the present test system. This factor, however, depends on the purity and activity of the preparation and should, thus, be experimentally established for each preparation. For example, the enzyme suspension furnished with the Boehringer test kit differed greatly from the preparation obtained from another commercial source. Based on the concentration of enzyme protein per milliliter, the kit preparation is a more dense suspension and apparently has an activity about five times that of the other commercially obtained preparation. Accordingly, the kit method requires only about 0.026 mg. enzyme protein per milliliter of reaction mixture, whereas the present method requires 0.12 mg. per milliliter of reaction mixture.

The present test system utilizes more DPN per milliliter of reaction mixture than other enzymatic methods. A lesser amount could be used with only a slight loss in sensitivity and without an apparent loss in accuracy. However, failure to obtain a stabilized DPNH concentration at the end of the incubation interval could lead to erroneous results. In this connection, Olson reported that maximum DPNH

formation occurred after 1 hour at 40° C. and after 2 hours at 25° C. An examination of the data upon which these conclusions were drawn, however, indicates that stability was not apparent at either temperature. Net absorption was continuing to increase after 2 hours at the lower temperature and appeared to be decreasing after 1 hour at the higher temperature. According to our observations, a greater quantity of DPN seems indicated for Olson's method.

The present study also establishes the usefulness of TCA filtrates. This factor seemed worthy of investigation since other ultraviolet-enzymatic lactate methods employ perchloric acid filtrates. The closely allied dye method of Friedland and Dietrich (8), however, utilizes TCA. Our interest in the preferential use of TCA was stimulated by two factors. First, the yield and clarity of PCA filtrates left much to be desired. Second, since other variables in our test battery were being analyzed from TCA filtrates, the preparation and use of a single filtrate for all analyses was desirable.

In terms of performance characteristics, the present method seems to offer several advantages over other enzymatic methods. With the elimination of certain procedural steps and the use of semimicro pipets and cuvettes, analyses may be carried out in a shorter period of time and with greater ease. This gain in simplicity is accomplished without a loss in precision. Further, the rather simple means employed for the drift correction would seem to provide a more realistic correction than that obtained by making multiple absorbancy readings of all blank and test samples or by using several reagent blanks. Also worthy of special note is the early plateau of net absorption values achieved with the present method. Olson reported that an incubation period of 2 hours at 25° C. was required for the complete oxidation of 0.2 μM. or less of L-lactate. Since the 50 mg. % lactate standard herein reported would correspond to 0.21 μM. of the L-lactate in the reaction mixture, and since the oxidation was complete within 35 minutes, it is apparent that the present test system achieves complete oxidation in less than one-third the time required by Olson's method.

Recovery data indicate that the described test system is a sensitive and reproducible method. Further, the comparative analysis of several blood filtrates by a well-known chemical method versus the enzymatic method establishes the accuracy of the latter.

REFERENCES

1. Horn, H. D., and F. H. Bruns. Biochim. Biophys. Acta 21:378 (1956).
2. Pfleiderer, G., and K. Dose. Biochem. Z. 326:436 (1955).
3. Hess, B. Biochem. Z. 328:110 (1956).
4. Scholz, R., H. Schmitz, T. Bucher, and J. O. Lampen. Biochem. Z. 331:71 (1959).
5. Hohorst, H. J. Biochem. Z. 328:509 (1957).
6. Boehringer, C. F., and Sons. Instruction Article No. 15-972, Mannheim, Germany.
7. Olson, G. F. Clin. Chem. 8:1 (1962).
8. Friedland, I. M., and L. S. Dietrich. Anal. Biochem. 2:390 (1961).
9. Barker, S. B., and W. H. Summerson. J. Biol. Chem. 138:535 (1941).

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-49. RAPID, ACCURATE ANALYSIS OF BLOOD LACTATE. June 1963, 8 pp. incl. illus., tables, 9 refs.

A method is described for the enzymatic analysis of blood L-lactic acid. The method is based on existing methods, but incorporates certain unique features which permit a rapid and precise analysis of a large number of samples. One of the more important features is the use of a simplified procedure for correcting the problematic drift of the unstable reaction mixture. In addition, conditions for the enzymatic activity were established which facilitate complete

1. Physiologic chemistry
2. Lactate analysis

I. AFSC Task 775801
II. J. P. Ellis, Jr., S. M. Cain, E. W. Williams
III. In ASTIA collection

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-49. RAPID, ACCURATE ANALYSIS OF BLOOD LACTATE. June 1963, 8 pp. incl. illus., tables, 9 refs.

A method is described for the enzymatic analysis of blood L-lactic acid. The method is based on existing methods, but incorporates certain unique features which permit a rapid and precise analysis of a large number of samples. One of the more important features is the use of a simplified procedure for correcting the problematic drift of the unstable reaction mixture. In addition, conditions for the enzymatic activity were established which facilitate complete

1. Physiologic chemistry
2. Lactate analysis

I. AFSC Task 775801
II. J. P. Ellis, Jr., S. M. Cain, E. W. Williams
III. In ASTIA collection

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-49. RAPID, ACCURATE ANALYSIS OF BLOOD LACTATE. June 1963, 8 pp. incl. illus., tables, 9 refs.

A method is described for the enzymatic analysis of blood L-lactic acid. The method is based on existing methods, but incorporates certain unique features which permit a rapid and precise analysis of a large number of samples. One of the more important features is the use of a simplified procedure for correcting the problematic drift of the unstable reaction mixture. In addition, conditions for the enzymatic activity were established which facilitate complete

1. Physiologic chemistry
2. Lactate analysis

I. AFSC Task 775801
II. J. P. Ellis, Jr., S. M. Cain, E. W. Williams
III. In ASTIA collection

USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-49. RAPID, ACCURATE ANALYSIS OF BLOOD LACTATE. June 1963, 8 pp. incl. illus., tables, 9 refs.

A method is described for the enzymatic analysis of blood L-lactic acid. The method is based on existing methods, but incorporates certain unique features which permit a rapid and precise analysis of a large number of samples. One of the more important features is the use of a simplified procedure for correcting the problematic drift of the unstable reaction mixture. In addition, conditions for the enzymatic activity were established which facilitate complete

1. Physiologic chemistry
2. Lactate analysis

I. AFSC Task 775801
II. J. P. Ellis, Jr., S. M. Cain, E. W. Williams
III. In ASTIA collection

oxidation of lactate in 30 to 35 minutes. A comparative analysis of several blood filtrates, using the present method versus a well-known chemical method, as well as recovery analyses using the two methods, establishes the validity of the present test system.

oxidation of lactate in 30 to 35 minutes. A comparative analysis of several blood filtrates, using the present method versus a well-known chemical method, as well as recovery analyses using the two methods, establishes the validity of the present test system.

oxidation of lactate in 30 to 35 minutes. A comparative analysis of several blood filtrates, using the present method versus a well-known chemical method, as well as recovery analyses using the two methods, establishes the validity of the present test system.

oxidation of lactate in 30 to 35 minutes. A comparative analysis of several blood filtrates, using the present method versus a well-known chemical method, as well as recovery analyses using the two methods, establishes the validity of the present test system.